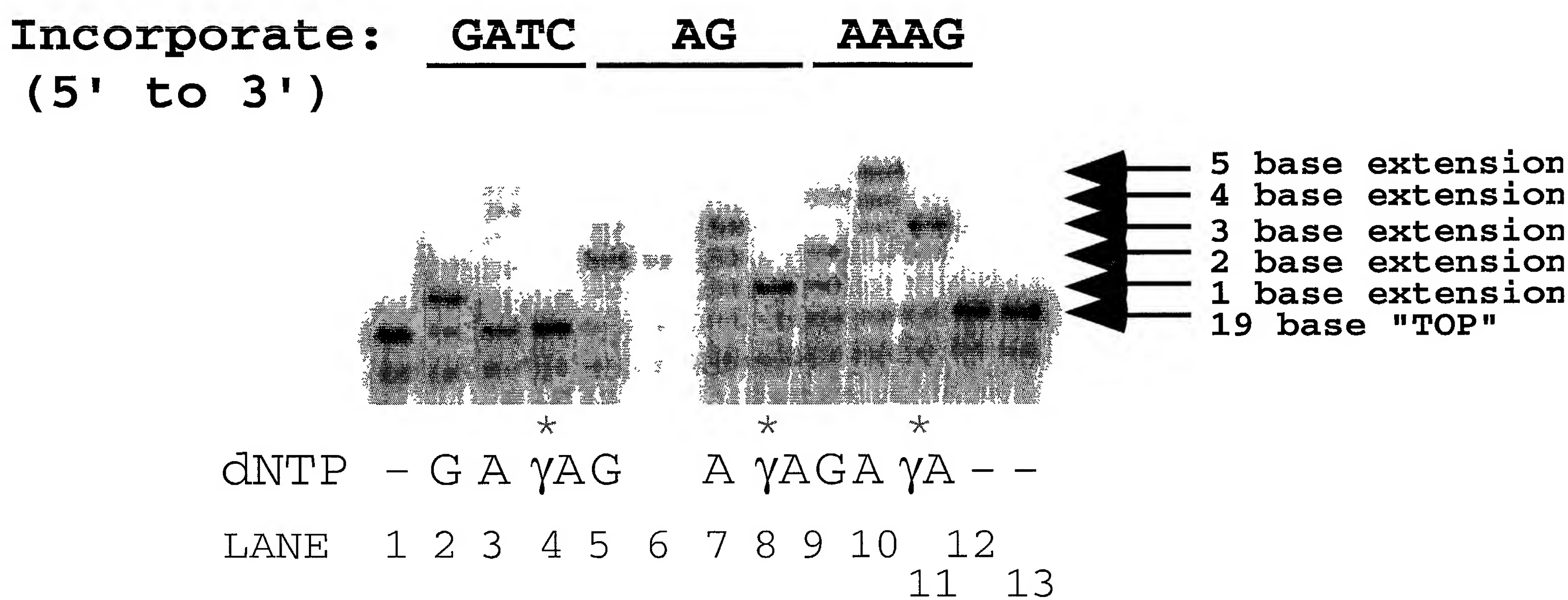
Fig.1



γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Fig.2

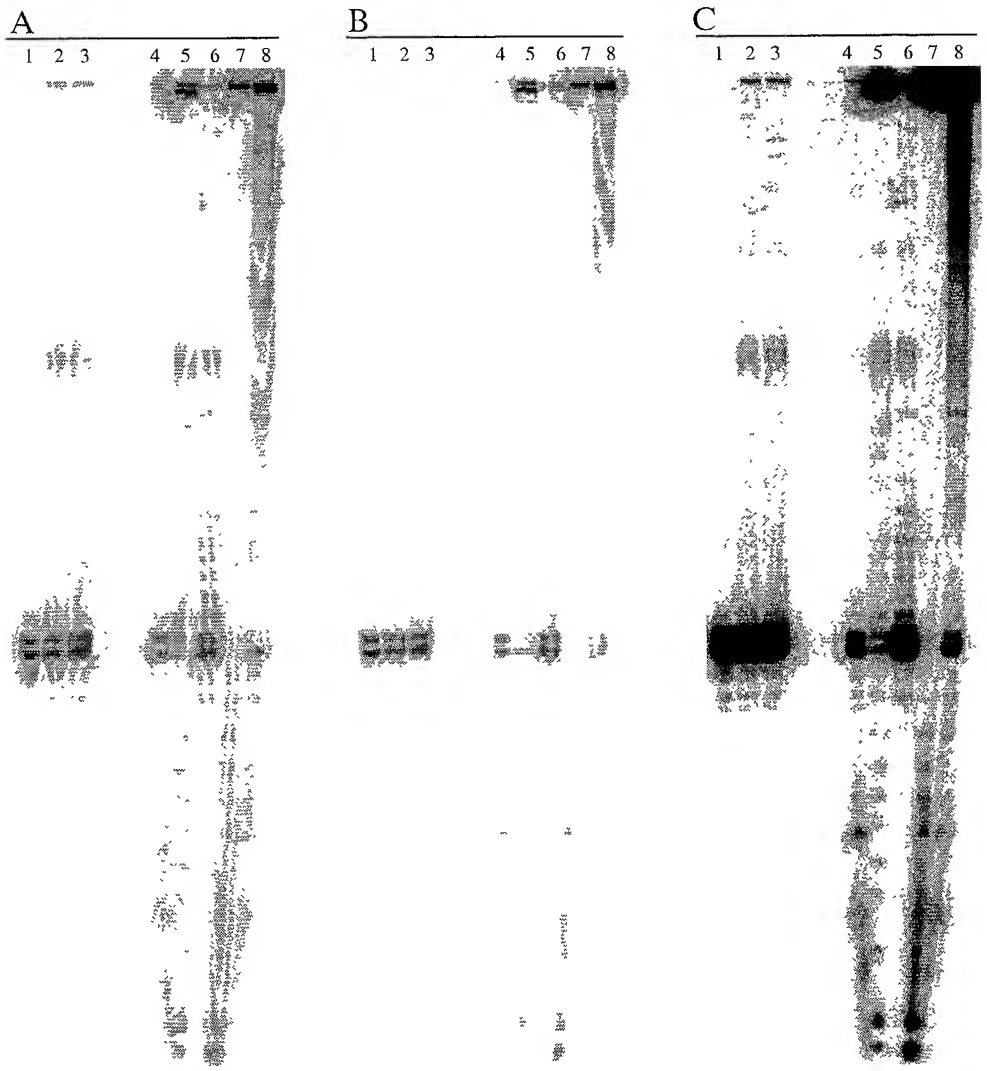


Fig.3

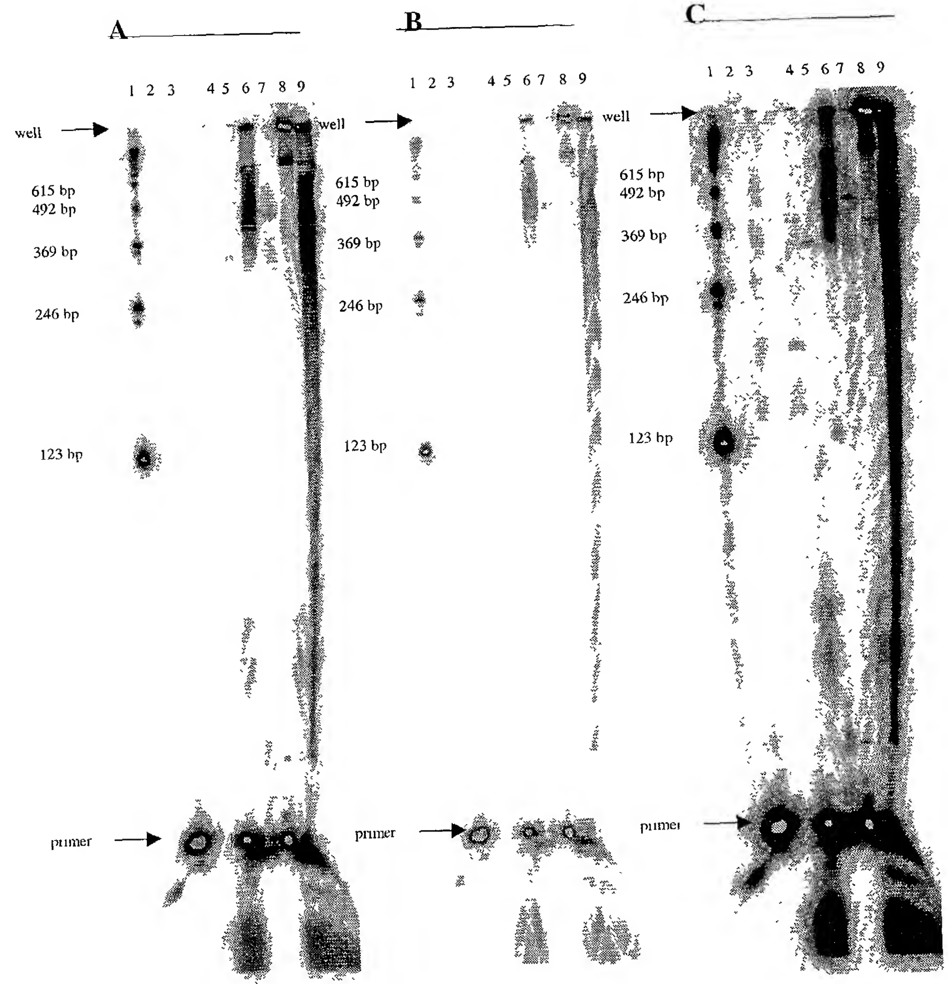
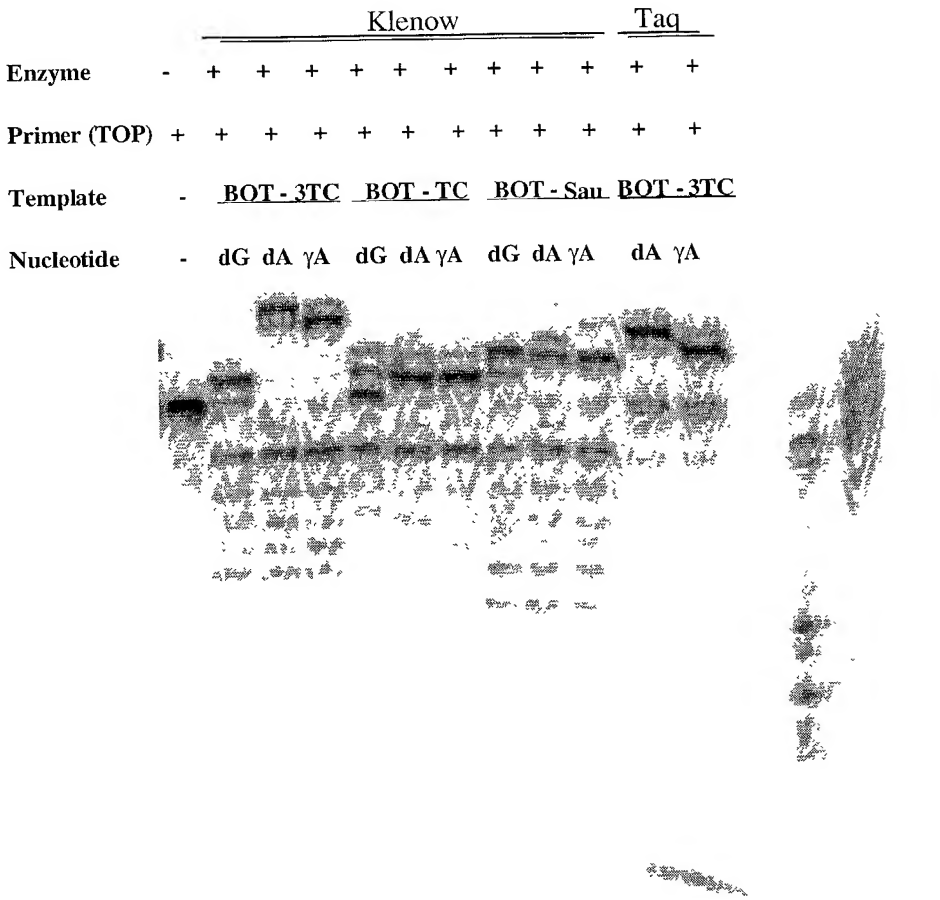
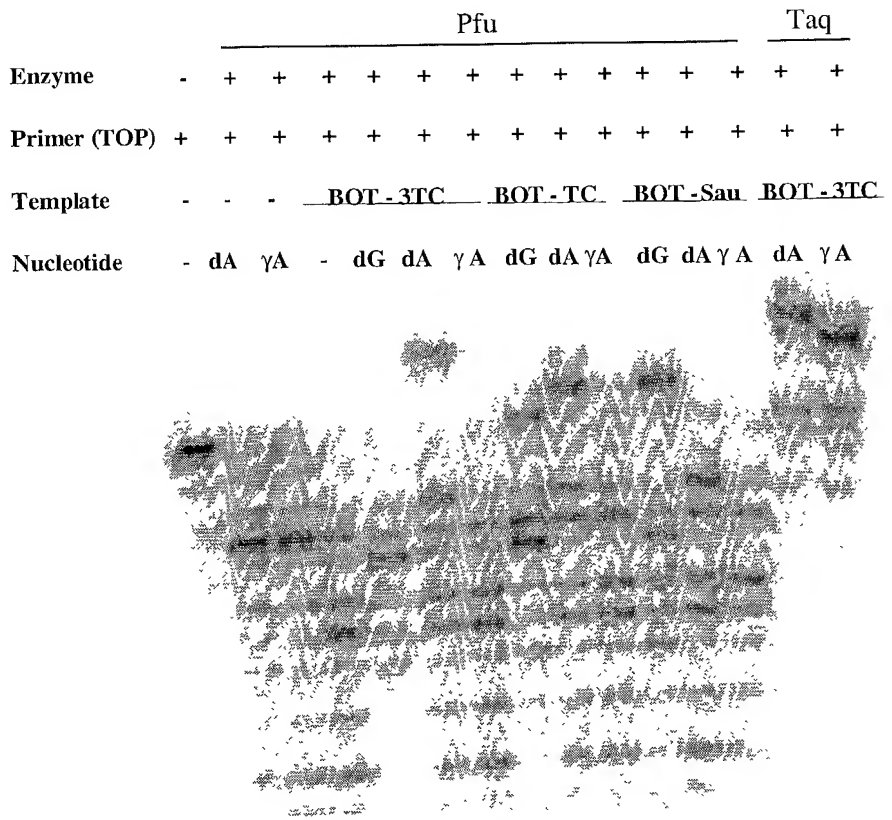

A
B
C
1 2 3 4 5 6 7 8 9
1 2 3 4 5 6 7 8 9
1 2 3 4 5 6 7 8 9
well
615 bp
492 bp
369 bp
246 bp
123 bp
primer
well
615 bp
492 bp
369 bp
246 bp
123 bp
primer
well
615 bp
492 bp
369 bp
246 bp
123 bp
primer

Fig.4



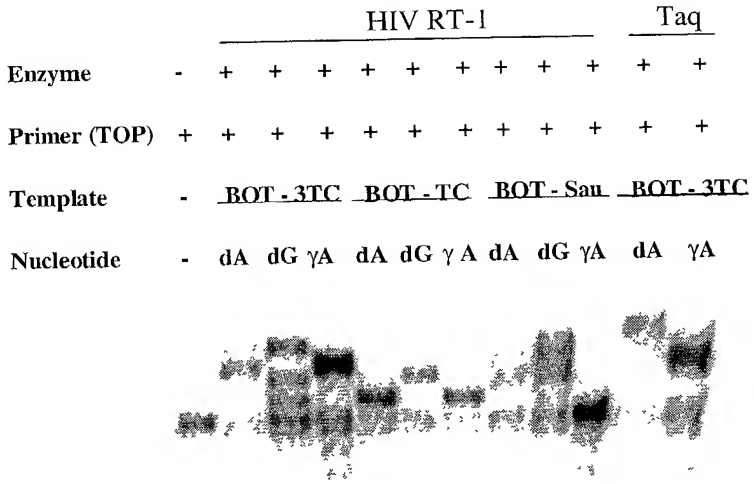
γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Fig.5



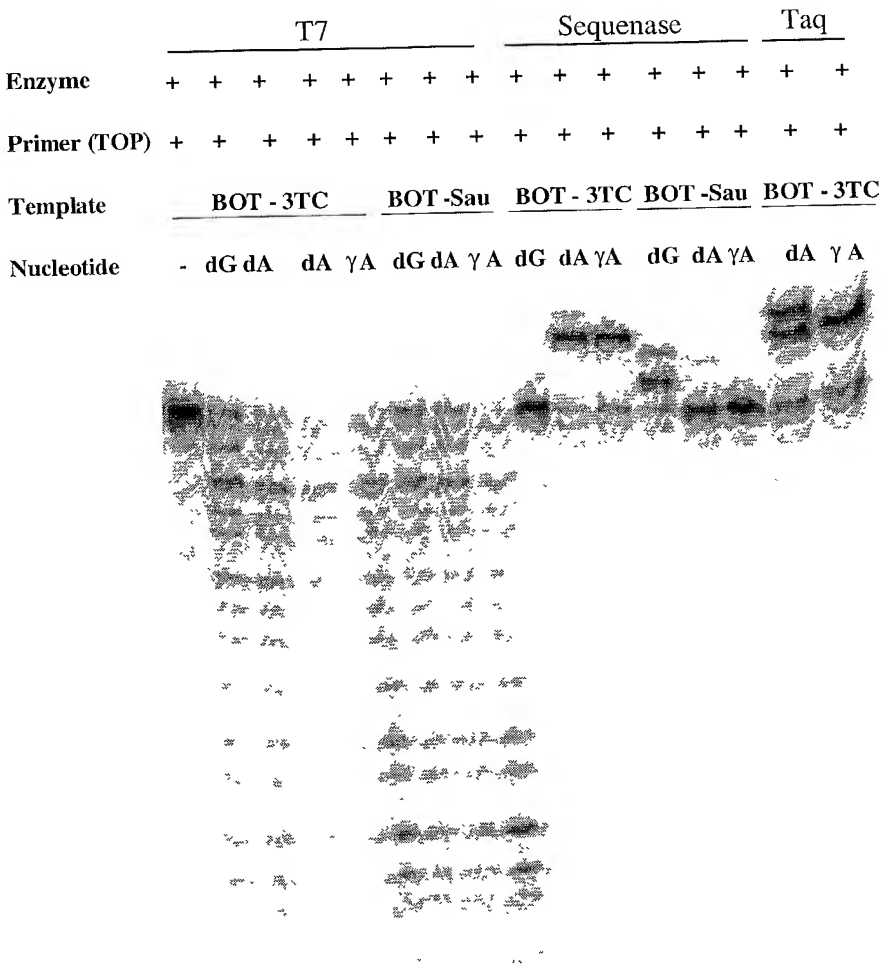
γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Fig.6



γ implies presence of an ANS-tag attached via the dNTP γ phosphate

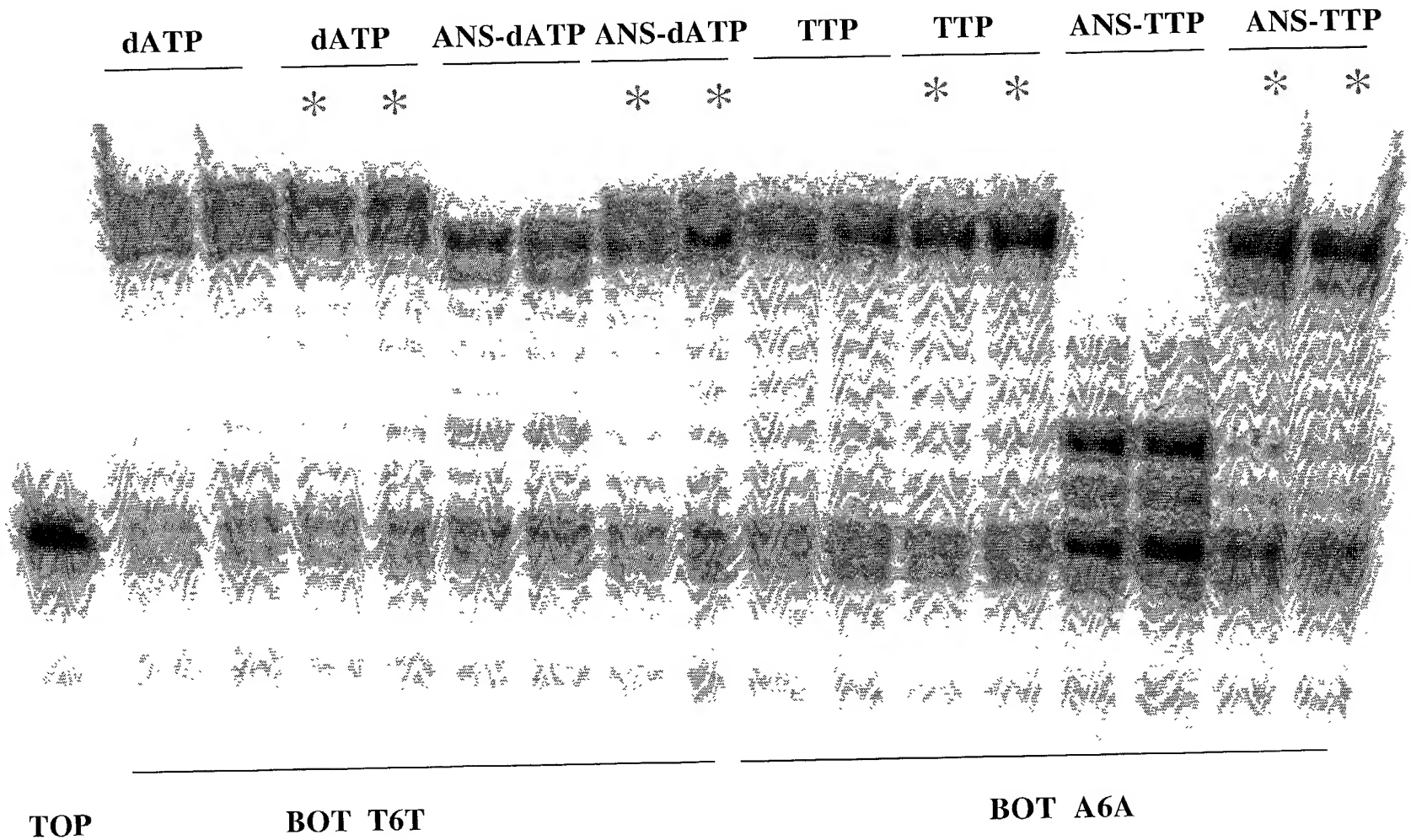
Fig.7



γ implies presence of an ANS-tag attached via the dNTP γ phosphate

Fig.8

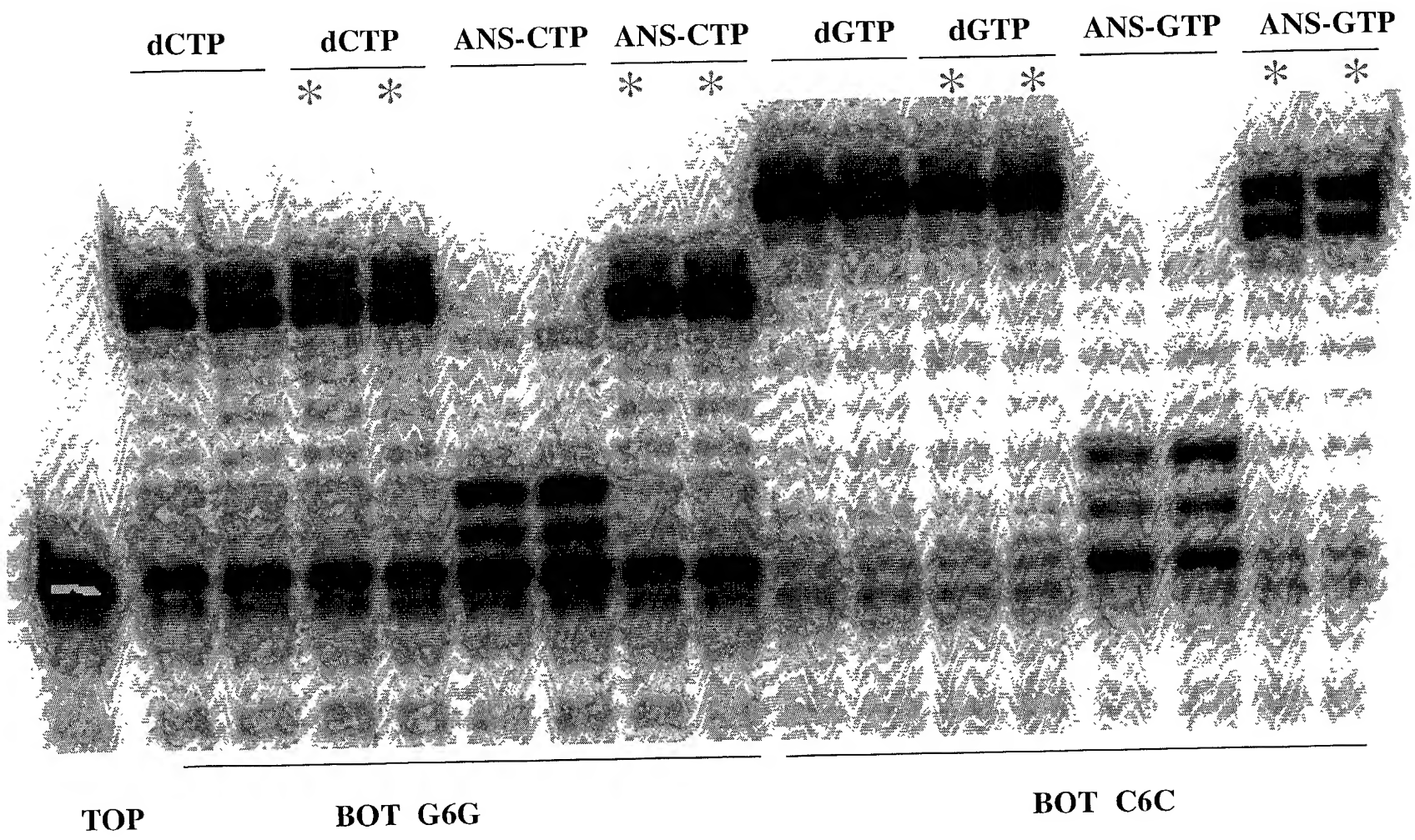
* - HEAT TREATED



10μM each dNTP; *Taq DNA Polymerase; extension 30' @ 37°C*

Fig.9

* - HEAT TREATED



10μM each dNTP; *Taq DNA Polymerase; extension 30' @ 37°C*

Fig.10

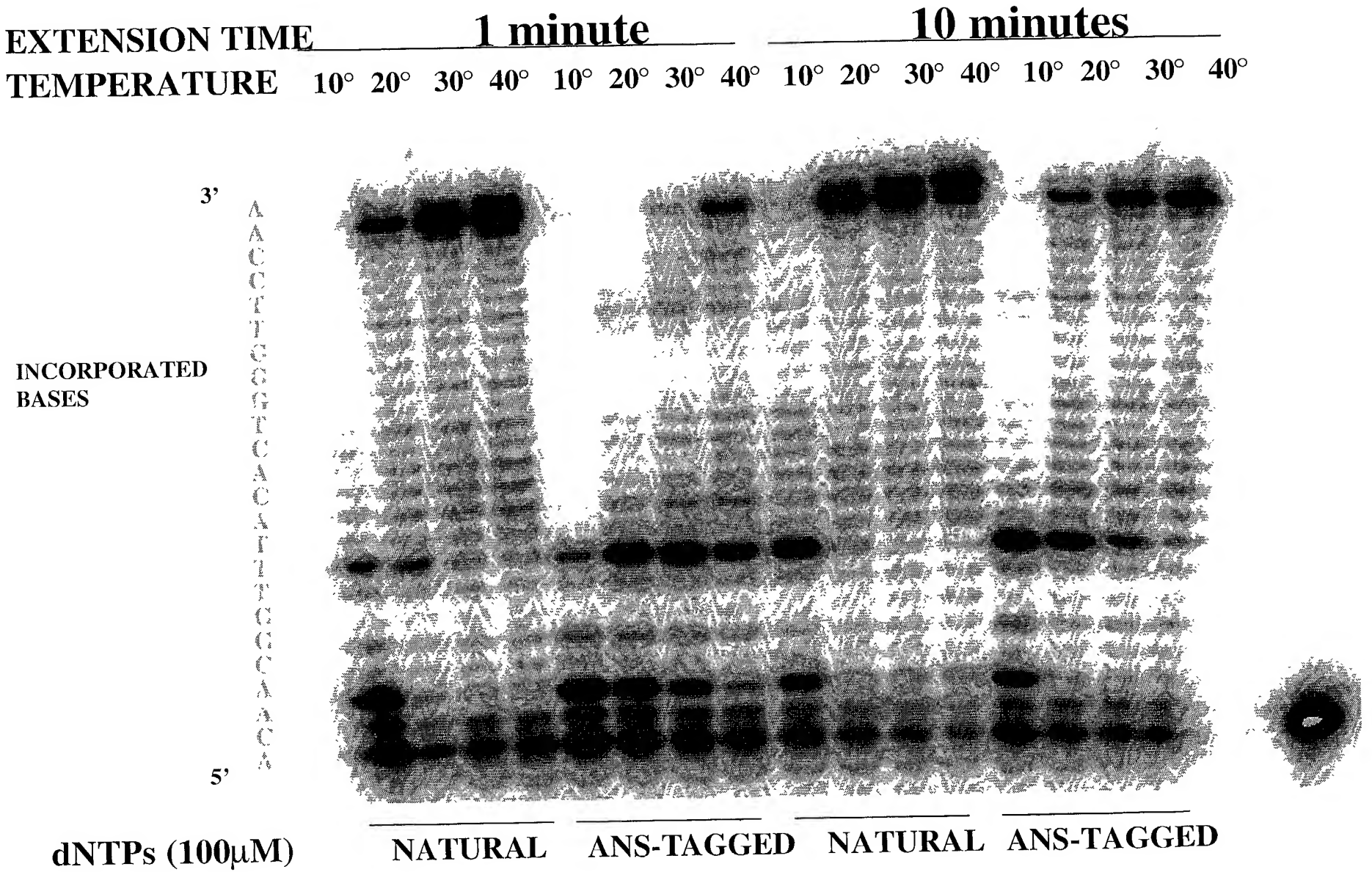
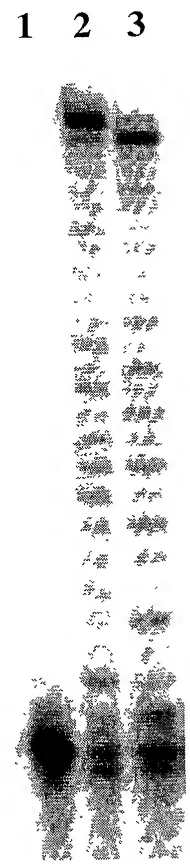


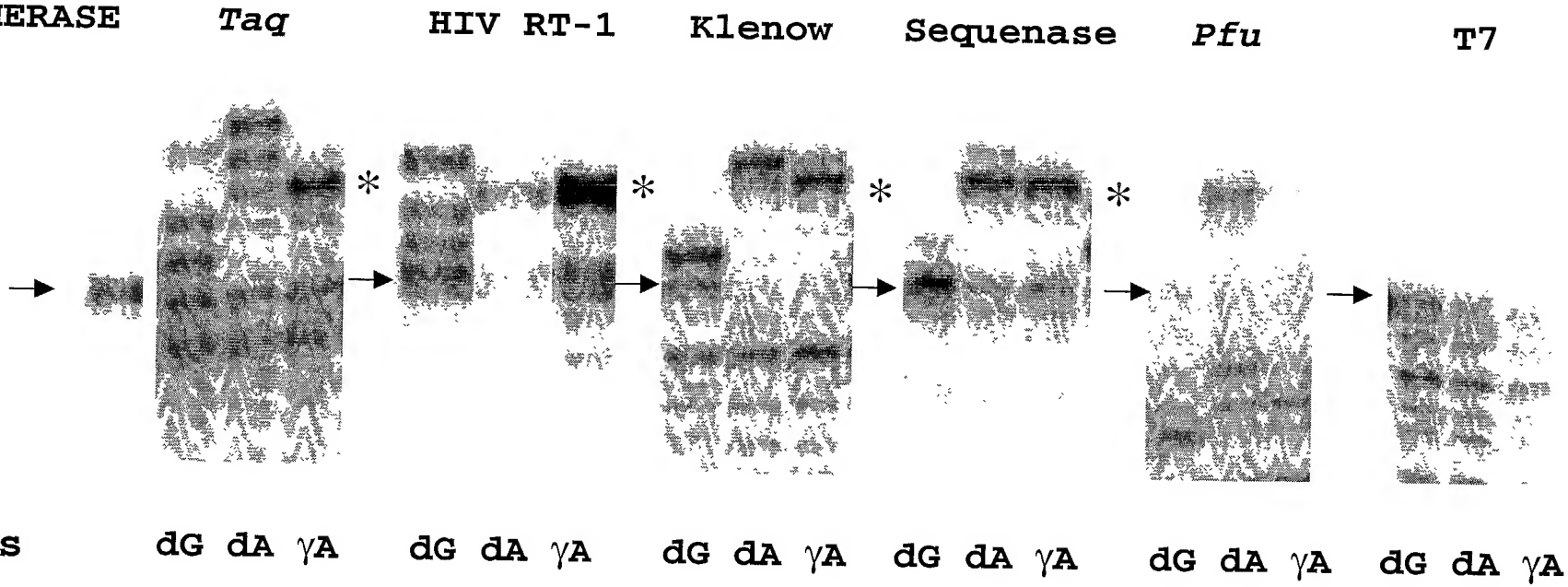
Fig.11

Primer sequence: 5' GGTACTAAGCGGCCGCATG 3'

Template sequence: 3' CCATGATTCGCCGGCGTAC<u>TGTTGCCAAATGTGACCCAAGGTT 5'</u>



Primer sequence: 5' GGTACTAAGCGGCCGCATG 3'
Template sequence: 3' CCATGATTCGCCGGCGTACTTTC5'



Different Polymerases React Differently to the ANS-γ-modified Nucleotides: primer extension reactions were performed to determine the ability of various polymerases to incorporate γ-tagged dNTPs during DNA polymerization.Control reactions contained natural dNTPs to monitor for template-directed nucleotide incorporation as well as for misincorporation. The reactions were performed in the appropriate buffer and contained the specified polymerase, primer/template duplex (radiolabeled 'TOP'primer annealed to 'BOT-3TC'template), and only the indicated dNTP. The reactions were carried out at room temperature or at 37°C for 30 minutes and were stopped by the addition of 0.5mM EDTA. The volume of the reaction was then reduced to approximately 2-4µl, loading dye was added and the polymerization products were electrophoresed through a 20% denaturing polyacrylamide gel.Arrows indicate the position of the free labeled 'TOP'. Asterisks indicate 3-base extension.

FIG. 12

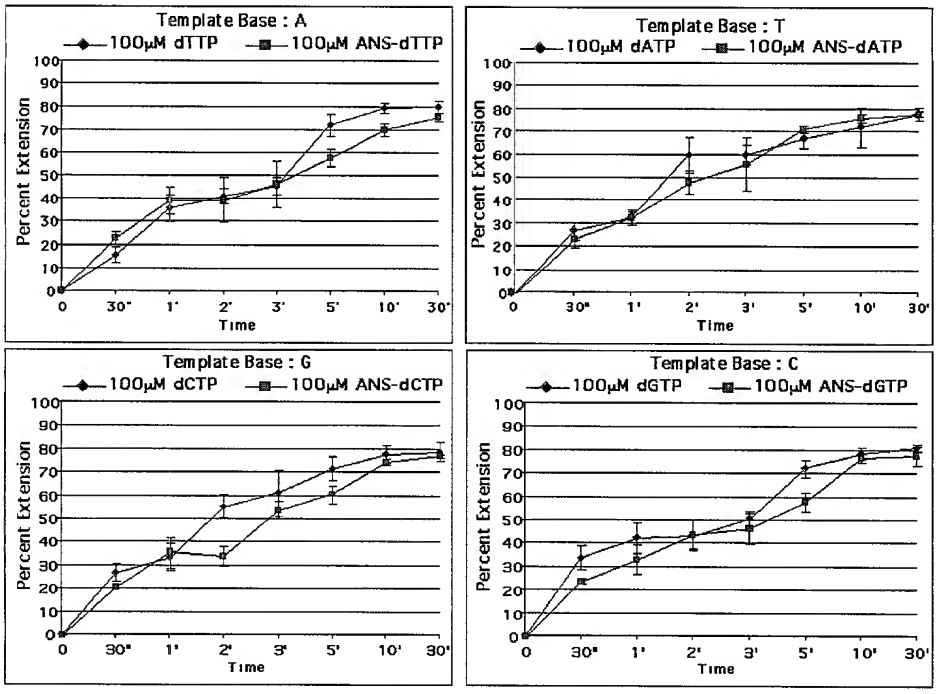
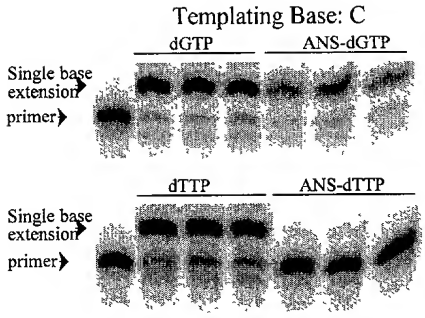

Template Base : A
100μM dTTP
100μM ANS-dTTP
Percent Extension
Time
Template Base : T
100μM dATP
100μM ANS-dATP
Percent Extension
Time
Template Base : G
100μM dCTP
100μM ANS-dCTP
Percent Extension
Time
Template Base : C
100μM dGTP
100μM ANS-dGTP
Percent Extension
Time

FIG. 13


Templating Base: C
dGTP
ANS-dGTP
Single base extension
primer
dTTP
ANS-dTTP
Single base extension
primer

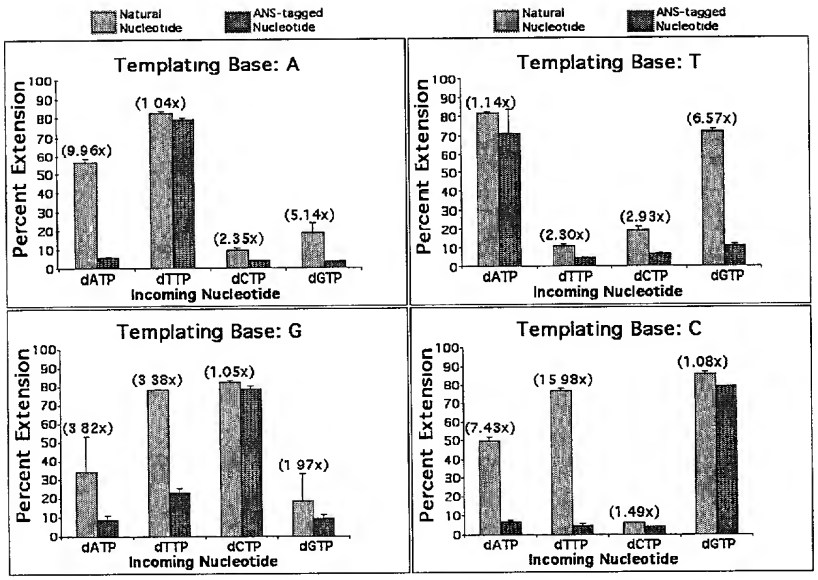

Natural Nucleotide
ANS-tagged Nucleotide
Templating Base: A
Percent Extension
(9.96x)
(1.04x)
(2.35x)
(5.14x)
dATP
dTTP
dCTP
dGTP
Incoming Nucleotide
Templating Base: T
(1.14x)
(2.30x)
(2.93x)
(6.57x)
Templating Base: G
(3.82x)
(3.38x)
(1.05x)
(1.97x)
Templating Base: C
(7.43x)
(15.98x)
(1.49x)
(1.08x)

FIG. 14